5-2014

Effects of Ketamine on Alcohol-Withdrawal Induced Behavioral Depression

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THE EFFECTS OF KETAMINE ON ALCOHOL-WITHDRAWAL INDUCED BEHAVIORAL DEPRESSION

by

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A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Biology)

The Honors College

University of Maine

May 2014

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ABSTRACT

The focus of this experiment was to test the possible anti-depressant effects of ketamine in a novel model of alcohol withdrawal-induced depression in mice. In this model, inbred mice show long-term strain-dependent reductions in locomotor (running wheel) activity after chronic intermittent ethanol exposure via vapor chambers. Since wheel-running is rewarding and has antidepressant effects, we believe that reduced locomotion following ethanol withdrawal may reflect a depression-like syndrome resembling that seen in many abstinent alcoholics. Nevertheless, in a previous experiment we were unable to reverse this locomotor deficit using Desipramine, a norepinephrine-selective reuptake inhibitor with behavioral activating properties. Since low-dose ketamine has demonstrated rapid-onset antidepressant effects in both human patients and animal models, we tested the ability of single ketamine injections to increase wheel-running in ethanol-withdrawn mice. Preliminary results indicate that ketamine was ineffective in this model, at least at the dose and treatment regimen employed.

ACKNOWLEDGEMENTS

Thank you to my precious family, especially Mom and Dad, for all of the support over the past 22 years. Thank you to my partner-in-thesis Jamie Ford and other lab members for their help throughout the project. Thanks to the Center for Undergraduate Research at the University of Maine for sponsorship of this thesis research. Special thanks to Alan Rosenwasser for countless edits and answered questions.

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INTRODUCTION

Alcoholism and/or harmful drinking affects an estimated 17 million Americans (CDC, 2009). Globally, it is the leading risk factor of premature death among persons 15-49 years old. Heavy drinking can lead to dependence, which in humans, is generally characterized by more than 12-14 drinks per week (NIAAA, 2013). Cessation of drinking once dependent, leads to a disorder called Alcohol Withdrawal Syndrome.

Alcohol withdrawal syndrome (AWS) is often characterized in a solely physiological manner. However, it is strongly associated with affective behavioral disturbances such as increased anxiety and poor mood. The severity of withdrawal varies greatly by individual. Furthermore, the temporal scale of alcohol withdrawal syndrome is broken down into three parts: acute, early abstinence, and protracted abstinence. Acute withdrawal refers to the first seven days post-withdrawal, early abstinence begins at the end of acute withdrawal continues up until eight weeks post-withdrawal, and protracted abstinence is any time after those eight weeks, lasting for years.

Acute withdrawal is the most widely studied area of alcohol withdrawal syndrome. In humans, this phase refers to the initial few days after the initial withdrawal until the seven days post-withdrawal. During this stage, the nervous system is hyperexcitable. Symptoms include tremors, autonomic nervous system instability, tremors, and seizures (Heilig, 2010). Disturbed sleep and circadian rhythms are also characteristic of acute withdrawal (Heilig, 2010). Additionally, acute alcohol withdrawal syndrome increases hyperventilation, anxiety, and craving for alcohol. The intensity of these symptoms is positively correlated to eventual relapse in protracted abstinence (Roeloffs, 1985).

Following the acute withdrawal period is early abstinence. Early abstinence is generally characterized by the weeks, up to eight weeks, following the initial withdrawal. In this stage, persistent anxiety, poor mood, and sleep disruption are very prevalent (Heilig, 2010). Anxiety levels have been show to decrease 3-6 weeks after the discontinuation of alcohol (Schuckit and Hesselbrock, 1994).

The third stage of abstinence, on the order of months to years after the initial withdrawal is protracted abstinence. Protracted abstinence is marked by affective instability. Trivial situations may initiate a very negative behavioral effect. The stress from these encounters triggers many alcohol abusers to crave alcohol and relapse (Sinha and Li, 2007). Sleep and circadian disruptions continue into this stage of alcohol withdrawal (Heilig, 2010). The intensity and persistence of these sleep and circadian disruptions is positively correlated to alcohol relapse (Drummond et al, 1998).

Most animal models have focused on the acute withdrawal period. In rodents, protracted abstinence, after chronic exposure to ethanol has a negative effect, just as humans. Alcohol-withdrawn mice lack novelty-seeking behavior and increase environmental habituation behavior, suggesting increased anxiety-like behavior. (Fukushiro et al, 2011). These studies also show that locomotor activity, as measured by the open-field test and home-cage running wheels, decreases after chronic ethanol exposure (Logan et al, 2010; Kliethermes et al, 2005). Some have argued that placing mice in a novel home-cage with a running wheel is, in fact, a measure of anxiety or fatigue, not behavioral depression (Stanford, 2007). This confound, though, is not a probable factor in running-wheel activity. In anxiety-like behavioral tests, very little reduction in baseline locomotor activity has been seen (Kliethermes, 2005). Therefore,

although there may be a relationship between anxiety and locomotor activity, it is one that will not contradict any observed information.

Neurobiology of Chronic Ethanol

It is now well-known that ethanol acts upon the $GABA_A$ and NMDA-glutamate receptors (Chandler et al, 1993; Mehta and Ticku, 1995; Mehta and Ticku, 1999). GABA is the most widespread inhibitory neurotransmitter. The $GABA_A$ receptor is a ligand-gated chloride ion channel. Activation of $GABA_A$ leads to inhibitory effects and contributes to inhibitory post-synaptic potentials (IPSP) (Sigel and Steinmann, 2012). On the contrary, glutamate is the most abundant excitatory neurotransmitter in the mammalian brain. The NMDA receptor is a ligand-gated cation channel and is the predominant mechanism for controlling memory function and synaptic plasticity (Fei et al, 2009). The NMDA-R is activated when it binds to glutamate *and* the membrane is depolarized; it is both voltage-gated and ligand-gated (Scheetz and Constantine-Paton, 1994). However, this can occur with the aid of α-amino-3-hydroxy-5-methylisoxazole-proprionic acid/kainate (AMPA/KA) receptors, another subtype of glutamate receptor. Often, they are in the same spatial and temporal range (Scheetz and Constantine-Paton, 1994). Furthermore, NMDA-R allows for the influx of calcium ions, activating localized calcium-dependent cascades and beginning the early steps of long term potentiation (Bliss and Collingridge, 1993).

Chronic ethanol exposure upregulates NMDA-R, in both increased function and number of receptors (Follesca and Ticku, 1995). Rani and Ticku (2006) cultured CIE neurons from rats and found that CIE exposure, and subsequent withdrawal, upregulated the subunits of NMDA-R, NR2B, but not NR1. Rani and Ticku suggest the CIE exposure

alters ion gating abilities, synaptic plasticity and transmission, hyperexcitability, and neurotoxicity. Chronic ethanol $GABA_A$ functions are downregulated via decreased mRNA expression (Rani and Ticku, 2006). Withdrawal from CIE induces $GABA_A$ receptor responsiveness to decrease extrasynaptically and increase in the synapses of the hippocampus (Liang et al, 2006). These effects may contribute to long-term alcohol dependence. The decrease in GABA neurotransmission during withdrawal directly inhibits dopaminergic neurons in the substantia nigra (Glue and Nutt, 1990). This inhibition leads to increase dopamine transmission, which may be correlated with increased severity of withdrawal symptoms (Heinz et al, 1996).

Chronic Intermittent Ethanol

Howard Becker and colleagues (Becker and Hale, 1993; Becker et al, 1997) developed a mouse model that represents alcohol dependence in mice. His studies use specialized chambers to force mice to become physically dependent on ethanol, since they will not otherwise become dependent on their own. Each individual chamber connects to a one-liter bottle of 95% ethanol, which then mixes into ethanol vapor and air in a tube, in order to force ethanol-saturated air into and out of the chamber. The levels at which ethanol vapor is pumped is determined by an air and ethanol gauge. Animals exposed to this protocol for six-to-nine days had the greatest effect when measuring withdrawal, by means of handling-induced convulsions (Goldstein, 1972).

Goldstein (1972) also found that in a 24-hour period, 16-hours of ethanol vapor, followed by 8-hours of fresh air, created the most intense withdrawal periods. This ethanol/air cycle, called the "kindling hypothesis," states that the period of detoxification allows for the severity of each subsequent withdrawal period to increase (Brown et al,

1988; Becker et al, 1997). Thus, by the end of the treatment, the mice have already experienced multiple withdrawal periods, making the final withdrawal period the most severe.

Logan et al (2010) used a chronic intermittent ethanol (CIE) procedure to determine the effects of alcohol withdrawal on circadian rhythms and locomotor activity. Logan exposed the C57/B6 mice to a four-day CIE protocol, in which each day consisted of 16 hours of ethanol vapor, followed by 8 hours of air. Throughout CIE exposure, mice were entrained to LD 12:12. At the end of four days, mice were placed into running-wheel cages in constant darkness. Logan discovered that there was a significant running-wheel deficit for seven days post-treatment, but resumed baseline activity on day eight. There was no significant change in circadian rhythmicity. The experiment suggested that the persistent effects of alcohol withdrawal induced depression may be quantifiable through running wheels and circadian rhythms.

Logan et al (2012), a follow-up study, mainly compared withdrawal-sensitive C3H mice to the B6 mice used in the previous experiment. He ran the C3H mice and B6 mice through 3 cycles of the 4-day CIE protocol, and additionally ran the C3H mice through one cycle of the protocol. Directly after CIE exposure, mice were placed into running wheel cages in constant darkness. Logan found that C3H mice showed reductions in running wheel activity for up to 30 days in both the 1-cycle and 3-cycle CIE treatment, whereas B6 mice displayed hypolocomotion for seven days in both treatments (Appendix A). C3H mice had significantly shortened free running periods, but B6 mice did not show any change in free running period. These findings, as Logan suggests, are likely due to genetic differences. The circadian alterations, moreover, may contribute to

the hypolocomotion, suggestive of behavioral depression. Lastly, his novel approach using running wheels as a motivational, reward-seeking measure of depression may be a simpler alternative to the current electrical stimulation.

Running Wheels

Previous data has shown that most laboratory mice will spontaneously run within five to ten minutes of first introduction to the wheels. Most mice will run regularly within five days of continuous exposure to the wheels, under otherwise normal conditions (Sherwin, 1998). Other contributing factors, such as age, oestrus, social conditions, and rearing environment, can be neglected, since all mice are approximately the same age (+/- 3 days), are male, and were housed in identical environments, with ample food and water supply.

In order to quantify a locomotor deficit, wheel-running can be used. Voluntary wheel-running is not just a generalized activity for mice (Sherwin, 1998); rather, it activates reward pathways within the mouse brain that have positive, lasting effects (Greenwood, 2011). Voluntary running-wheel activity in mice is comparable to voluntary exercise in humans- improving physical and mental health conditions (Eikelboom, 1998). Often, drugs of abuse initiate the same reward pathways, leading to addiction and depression. Running wheels, in mice, have been shown to reverse this effect, thus can be considered an antidepressant activity (Brene et al, 2007). Moreover, running-wheel activity is shown to be anxiolytic, across several anxiety measures, such as light-enhanced startle, open-field test, stress-induced hyperthermia, social interaction (Salam et al, 2009). Based upon this evidence, low running wheel activity can be

construed as a measure of behavioral depression, analogous to human behavioral depression.

Two strains of mice are typically used in our lab for ethanol experiments: C3H/HeJ and C57/B6. The mice we chose, strain C3H/HeJ, are prone to anxiety-like behaviors, as shown through a variety of standardized tests (Milner and Crabbe, 2008). During ethanol exposure, they show high blood ethanol concentration, relative to B6 mice (Metten and Crabbe, 2005). Throughout withdrawal periods, C3H have severe handling-induced convulsions (HICs), also referred to as seizures, the second highest of fifteen tested strains (Metten and Crabbe, 2005). The C3H mice are much less alcohol-preferring and have more severe withdrawals, consequently, making them the ideal strain for this experiment.

Antidepressant Treatments

Previous studies in our lab have shown that mice exposed to the chronic intermittent ethanol (CIE) protocol, followed by subsequent withdrawal, show decreased locomotor activity, as measured through the use of running-wheels (Logan et al, 2012) (Appendix A). The induced hypolocomotion is interpreted to be a state of behavioral depression, analogous to human depression. Therefore, it would be logical to investigate possible treatments, namely, clinical antidepressants. If the antidepressants were to work, we could then expect to see an increase in running-wheel activity of the treated animals.

Prior to the current experiment, we tested the effects of desipramine on alleviating alcohol-withdrawal induced depression. Desipramine is a tricyclic antidepressant (TCA) and is commonly used to treat endogenous depression. It inhibits the reuptake of norepinephrine and, to a small extent, serotonin (Mayo Clinic Laboratories, 2014). In

humans, Desipramine usually takes about two or three weeks to take effect. In mice, desipramine has been linked to improving depression-like states, such as cytokine levels (Kubera et al, 2001). In our laboratory's tests, we were unable to see a significant difference in running-wheel activity when ethanol-withdrawn mice were treated with desipramine (Rosenwasser et al, unpublished). We concluded, then, that desipramine neither alleviated nor reversed the effects of alcohol withdrawal induced depression.

Another type of antidepressant has been of recent interest to researchers: ketamine. Ketamine is a street drug and a veterinary anesthetic. The drug was created in 1962 to be used as a fast-acting, battlefield anesthetic (Jansen, 2000). Since then, it has made its way onto the illicit drug scene, where it can produce effects such as hallucinations, increased libido, elevated heart rate, and euphoria (Jansen, 2000). Today, ketamine is largely used as an animal anesthetic, when administered in high doses. At mid-dosage levels, it is psychotomimetic. More recent studies, however, show that at very low doses, ketamine may be used as a putative antidepressant.

Much is unknown about ketamine. Preliminary studies have shown that injections with low doses of ketamine (0.5mg/kg) may alleviate major depression disorder in patients who are unresponsive to common treatment (Murrough et al, 2013). These patients responded to treatment within two hours of administration and lasted up to two weeks (Liebrenz et al, 2009). Furthermore, patients showed low chance of relapse of depression, up to three months (Murrough et al, 2013). Ketamine acts as a N-*methyl –D-aspartate* (NMDA) glutamate antagonist. Mice that develop helplessness (via learned helplessness, forced swim test) and subsequently treated with low dose ketamine show acute, yet sustained non-depression (Maeng et al, 2008). With all things considered,

treating mice undergoing alcohol withdrawal with ketamine may serve to alleviate the hypolocomotion associated with alcohol withdrawal.

Circadian Rhythms

Daily biological rhythms are run by an intrinsic clock. These rhythms are referred to as circadian rhythms and are driven by a biological clock. These rhythms unify almost all organisms, from unicells to brain cells (Lakin-Thomas and Brody, 2004; Welsh et al, 1995). The study of them has emerged into a field of chronobiology. The daily period, usually a twenty-four hour period, can be stimulated to induce a phase change with stimuli (Brager et al, 2010). Recent interactions in human and animal research propose interactions between alcohol exposure and/or withdrawal with disruptions in the sleep and the circadian clock. Human alcoholics, in extended periods of protracted withdrawal, have been shown to have persisting chronobiological disruptions, leading to relapse (Drummond et al, 1998; Brower, 2003). Chronic intermittent ethanol (CIE) exposure fundamentally alters the circadian clock, as measured by free-running period and response to phase-shifting stimuli (Rosenwasser et al, 2005a,b).

Rationale

In humans, glutamate may play a large role in the pathophysiology of major depression disorder (MDD). Studies have shown that glutamate levels increase in the cerebral spinal fluid (Levine et al, 2000) and increase in the brain (Hashimoto et al, 2007; Sanacora et al, 2004). Post-mortem protein assays have shown that the level of NMDA and AMPA receptors also decreased in the perirhinal cortex, though not the hippocampus or entorhinal cortex, in patients with MDD (Beneyto et al, 2007). Rodent models show similar results to that of humans. Mice characterized as “depressed,” by forced swim tests

and tail suspension tests in glutamate knockout mice, showed upregulation of the mGlu1 subunit of the NMDA-R and downregulation of mGluR2,3,4, and 7 (Tokita et al, 2012). Moreover, "depressed" rats showed decreased levels of NMDA-R in the many regions of the brain, including the cerebral cortex, amygdala, hypothalamus, hippocampus, and prefrontal cortex (Tokita et al, 2012). Reversely, both rats and mice were "depressed" when given an NMDA-R knockout, using various genetic mutations (Tokita et al, 2012).

Ketamine acts as a non-competitive NMDA-R antagonist. At subaneshetic levels, it is a putative antidepressant. In mice, it reduced "depression" symptoms, as tested by learned helplessness and forced-swim tests (Tokita et al, 2012). Ketamine prevents the excessive influx of calcium, thus, preventing cellular damage. The drug enhances the firing rate of glutaminergic neurons, in addition to the increased presynaptic release of glutamate (Moghaddam et al, 1997). In low doses, ketamine increases dopamine release in the prefrontal cortex, but this upregulation is secondary to the activation of AMPA/KA (Moghaddam et al, 1997). Furthermore, Moghaddam suggest that AMPA/KA activation may enhance the antagonistic effects of ketamine on NMDA-R. Ketamine-induced upregulation of AMPA/KA- glutamate receptor may allow rapid intracellular signaling and greater synaptic plasticity (Duman and Voleti, 2011). Another proposed mechanism and target of the antidepressant treatment is increased expression of brain-derived neurotrophic factor (BDNF) and the subsequent mRNA. The BDNF promotes neuroplasticity, neurogenesis, and neuroprotection, all which enhance the antidepressant effects (Duman and Voleti, 2011).

Ethanol acutely antagonizes NMDA receptors. Ethanol withdrawal has biphasic effects on glutamatergic transmission that may account for the central nervous system

hyperexcitability during acute withdrawal and the persistent depression associated with protracted abstinence.

Due to ketamine's ability to upregulate AMPA/KA-glutamate receptors and the hyperexcitable state of the central nervous system during protracted ethanol abstinence, there may be a possibility of ketamine counteracting the effects of ethanol withdrawal. We hypothesized that ketamine may be effective in preventing or alleviating the effects of withdrawal-induced behavioral depression.

MATERIALS AND METHODS

Subjects and Apparatus

Upon arrival in the laboratory, six to eight week old male C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) were weighed and housed in groups of five in standard mouse cages. Food (ProLab RMH3000, Lab Diet, St. Louis, MO) and tap water were readily available. Cages were placed in large (60x36x60cm) Plexiglass vapor inhalation chambers, two cages (ten mice) per chamber.

Procedures

Half of the mice (n=20) were exposed to an eight-day chronic intermittent ethanol vapor, while the other half (n=20) were exposed to eight days of plain air. All mice were maintained under LD 12:12 conditions throughout the CIE protocol. Twenty-four hours after the last exposure to ethanol, animals were injected with single dose of 10mg/kg, i.p., ketamine or saline.

Thereafter, mice were placed into individual cages, equipped with running-wheels (diameter 35cm). Each running-wheel cage was housed in a sound-and-light protected enclosure with a standard light bulb. Acclimation to wheel-running was measured and analyzed using ClockLab (Actimetrics Co., Wilmette, IL). All mice were maintained under constant darkness, in order to measure the free running period. The free running period is using daily wheel turns. Mice continued to have free access to tap water and food.

Chronic Intermittent Ethanol

The CIE protocol is based on the research of Becker et al. (1997) and Logan et al (2010). The protocol consists of eight daily cycles of 16 hours of ethanol vapor exposure followed by 8 hours of plain air exposure. The onset of ethanol vapor exposure was concurrent with the onset of 12 hours of darkness (LD 12:12). Ethanol vapor exposure ended four hours into the light cycle. Control mice were exposed to plain-air for all eight-days, but remained on the same LD 12:12 cycle as the CIE mice. Ethanol (95%) was pressurized into vapor and constantly pumped at a rate of 10-12 L/min (Logan et al, 2010).

Directly before each ethanol exposure, experimental animals were administered an injection with 1.6mg/kg of ethanol, and 68.1mg/kg pyrazole HCl. Pyrazole is an alcohol dehydrogenase inhibitor that initiates intoxication and stabilizes blood ethanol concentration (BEC) (Becker and Hale, 1993). Pyrazole was dissolved in 20% w/v ethanol solution and administered i.p. at 10mL/kg. Control animals were injected with the same quantity of pyrazole dissolved in a 0.9 % saline solution.

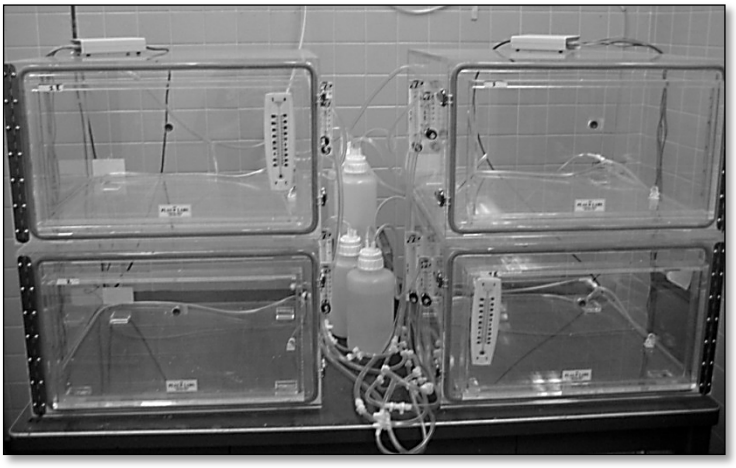

Image 1: Vapor Chambers. Mice exposed to CIE protocol were housed in chambers above. Each chamber connects to a pump that regulates the alcohol and air mixture input.

Ethical Considerations

This experiment was conducted with approval of the University of Maine Institutional Animal Care and Use Committee (IACUC).

RESULTS

Figure 1, below, shows the gradual increase of running-wheel activity in all groups of mice five weeks after final CIE treatment. Activity levels were expressed as wheel-turns per day and subjected to a three-factor analysis of variance (ANOVA), with ethanol treatment (vs. air), ketamine treatment (vs. saline) and post-treatment week as factors. An initial analysis using all five post-treatment weeks showed a significant effect of weeks ($F_{4,132} = 7.898$, $p < 0.001$) indicating that activity levels increased over time, but no effects of either ethanol or ketamine treatments. Nevertheless, a follow-up analysis restricted to the first three post-treatment weeks revealed a main effect of week ($F_{2,72} = 6.747$, $p = 0.002$) and a weeks-by-ethanol interaction ($F_{4,132} = 4.116$, $p = 0.002$). This result indicates that, as expected, activity levels increased more slowly in ethanol-treated than in air-exposed mice over the first three post-treatment weeks. Finally, we performed separate two-factor (ethanol, ketamine) ANOVAs for each post-treatment week, which showed a significant main effect of ethanol only at week 3 ($F_{1,36}= 4.435$, $p = 0.042$).

The free-running period is the endogenous period of time (typically 24.0 hours) that the steers the mouse's circadian rhythms. Baseline for these animals is determined by the control, which has a free-running period of approximately 23.7 hours (figure 2). Animals exposed to CIE treatment had a period of approximately 23.9 hours. Mice treated with only ketamine had an increased free-running period that nearly reached 24.2 hours, whereas mice treated with ketamine and ethanol had period between standard air and standard ethanol, at 23.8 hours. This suggests that although there was an effect of ketamine in the air animals, it did not alleviate any effects of alcohol withdrawal in the

ethanol treated mice. A two-factor (ethanol, ketamine) ANOVA failed to detect any significant treatment effects on free-running circadian periods.

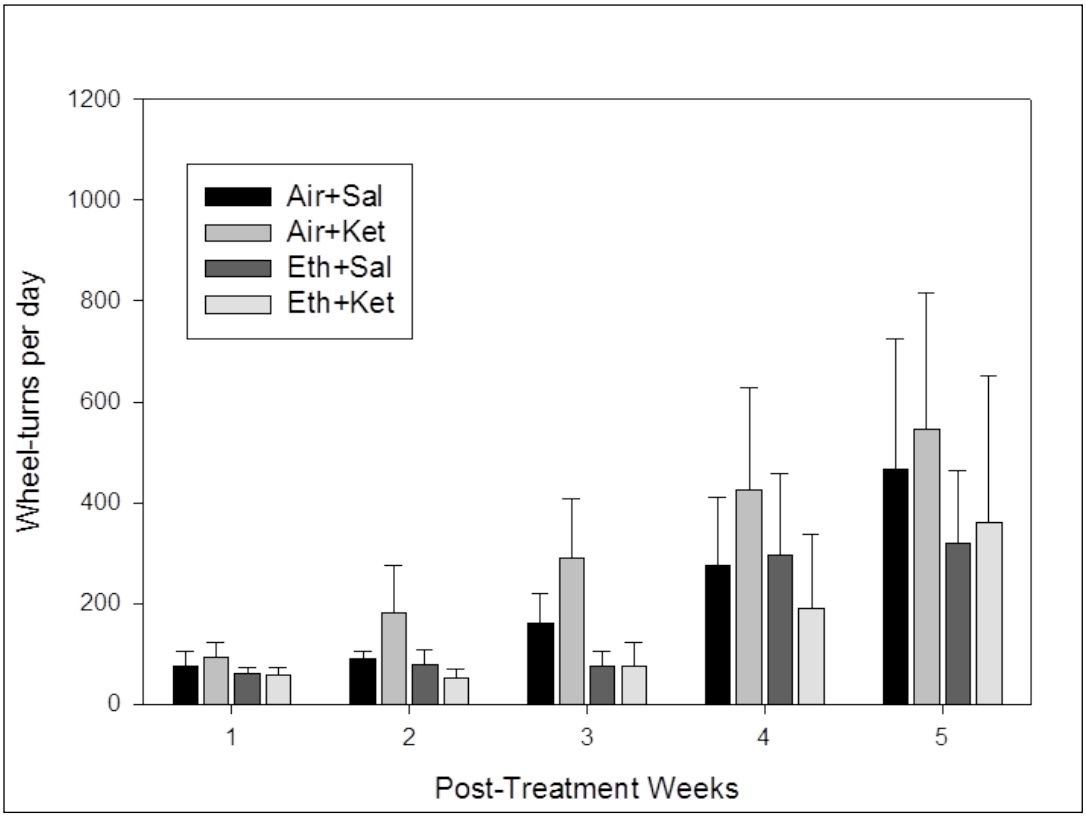

Figure 1. C3H Acclimation to Running Wheel Activity. All display an increase in locomotor activity over the course of five weeks post-treatment weeks, as expected. There is a significant decrease in ethanol-treated animals ($p=0.02$) over the first post-treatment weeks. Ketamine does not alleviate symptoms of ethanol-withdrawal.

Furthermore, the initial phase shows the endogenous circadian phase, measured in the first 24 hours of constant darkness. animals treated with saline and air have an initial phase of approximately 15 hours, whereas animals treated with saline and ethanol have an initial phase of approximately 16 hours (figure 3). This phase shift is expected due to the sleep-disrupting effects of ethanol withdrawal. Ketamine treated animals, however, display reverse effects. Animals treated with ketamine only had an initial phase of nearly

18 hours. On the opposite end of the spectrum, animals treated with CIE, followed by a ketamine injection, had an initial phase of about 11 hours. Two-factor (ethanol, ketamine) ANOVA revealed a significant ethanol-by-ketamine interaction ($F_{1,36}$= 4.504, p = 0.041). This interaction was examined using post-hoc pairwise comparisons which showed that initial phase was significantly earlier (advanced) in the ethanol+ketamine group relative to all other groups. This finding suggests that ketamine induced significant circadian phase-shifts only in ethanol-treated animals.

Figures 4 and 5 show a sample of daily running-wheel activity shifts over the six week length of the experiments. Ethanol-treated animals display a delayed phase shift lasting approximately three weeks post-initial withdrawal. After three weeks, the ethanol animals show the same phase shift the air mice did three weeks prior.

Throughout the CIE protocol, weights were measured every two days. Both groups, ethanol and air, lost small amounts of weight during the eight-day procedure (air: -1.625 grams; ethanol: -1.940 grams).

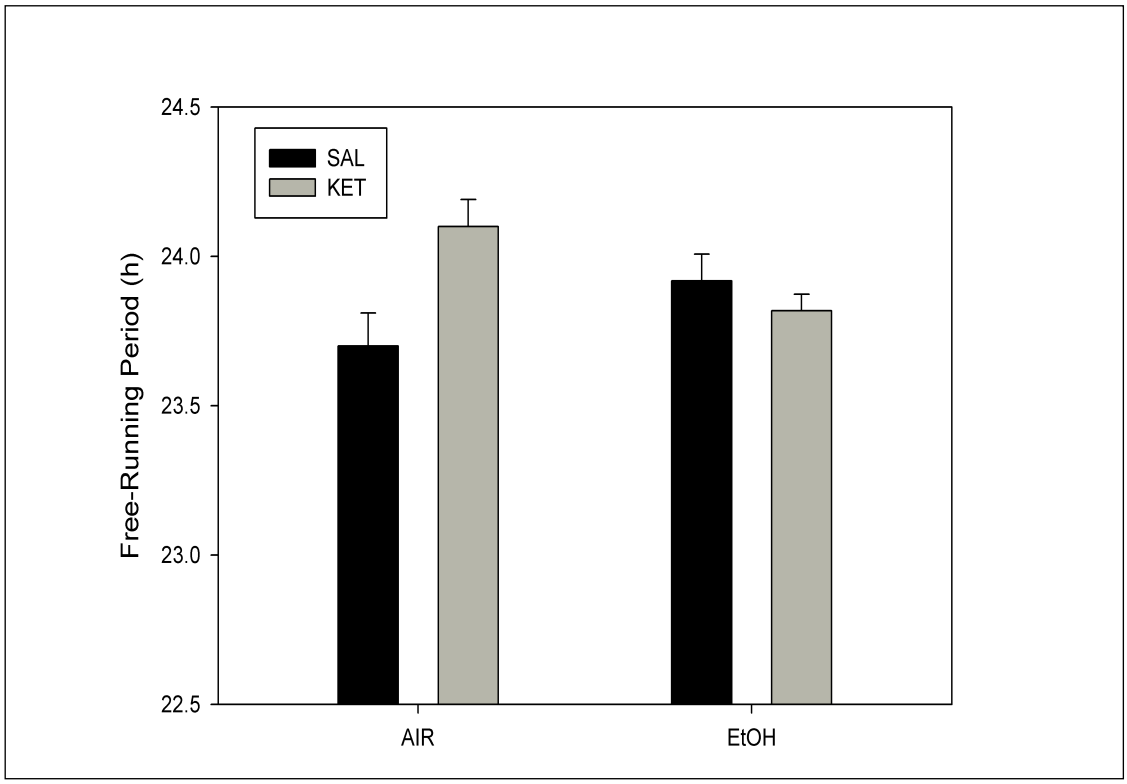

Figure 2. Free-Running period in Ethanol- and Ketamine-treated C3H mice. Mice exposed to CIE protocol, on average, had a slightly longer free-running period after the exposure. Mice exposed to air and ketamine had a longer period than all other groups combined.

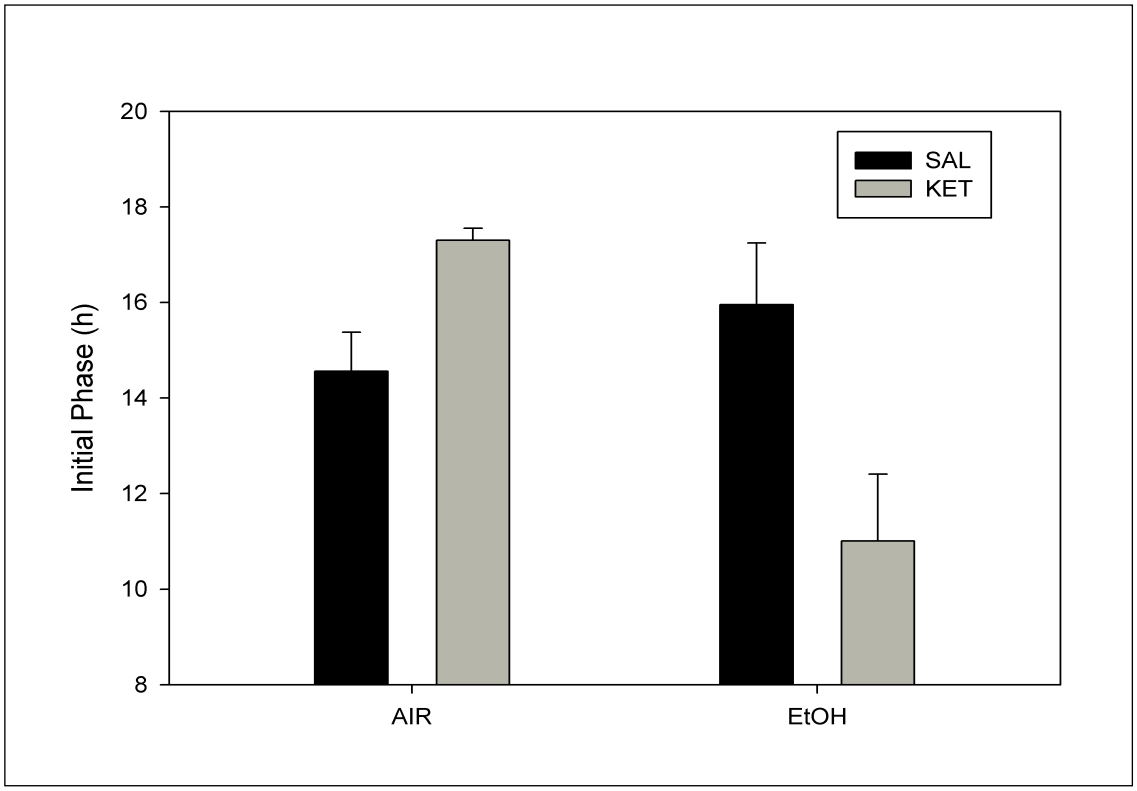

Figure 3. Initial Phase of Ethanol- and Ketamine-treated C3H mice. The initial phase of CIE-exposed mice is slightly higher than those exposed to air. However, there is a large effect of ketamine, in both air and ethanol exposed mice. Ketamine, when injected into air animals, greatly increases the initial phase. On the contrary, when injected into ethanol-exposed animal, ketamine reduces the initial phase by approxiamtely 25%.

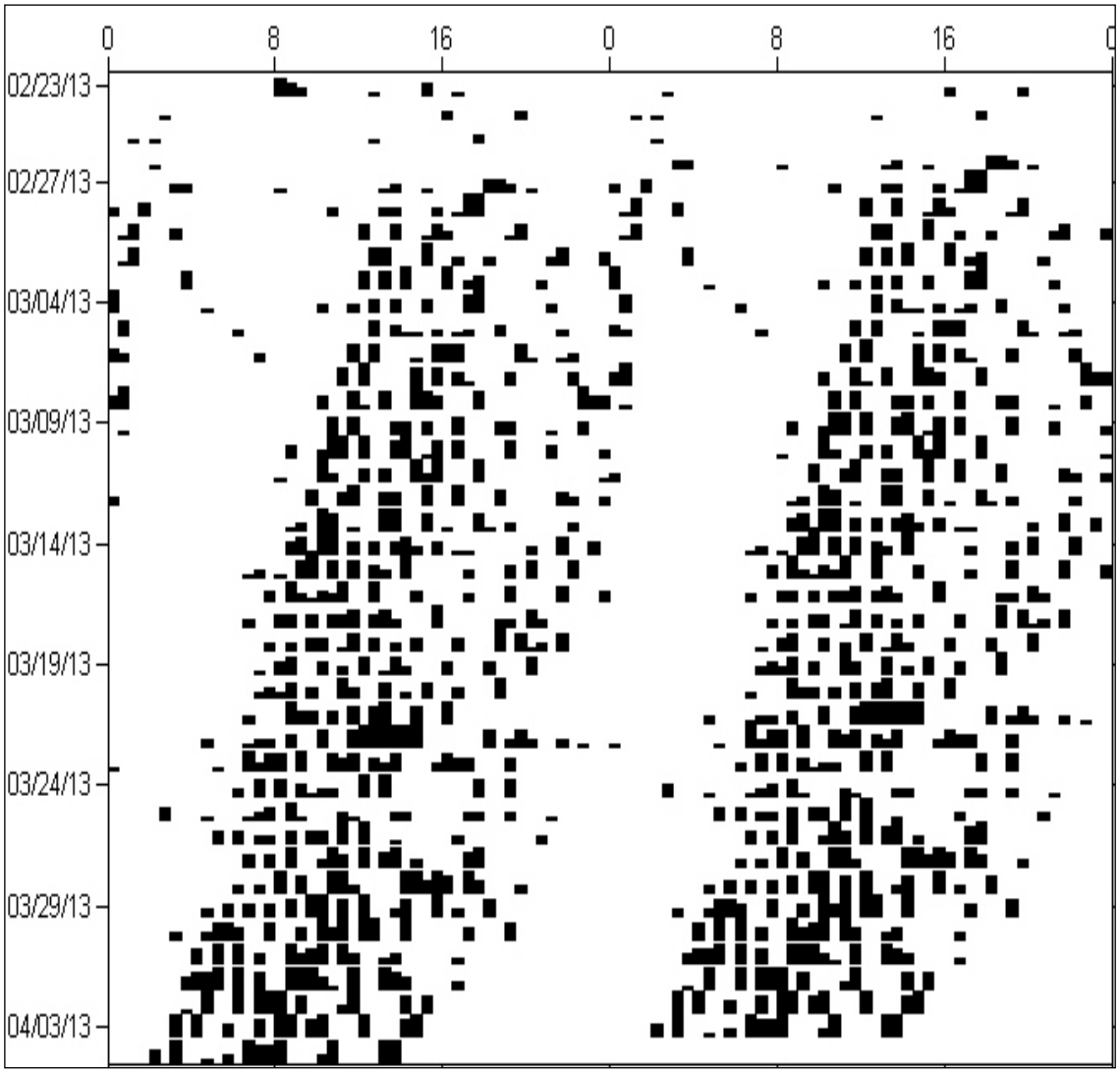

Figure 4. Air-exposed mouse actogram. Thissample actogram shows the gradual acclimation to running-wheels in air-exposed control animals. The running-wheel activity is initially sparse, but gradually increases over six weeks. Their phase slightly changes over time, suggseting normal circadian rhythmicity. (Note: X-axis represents time in hours, with each zero starting the next consecutive day).

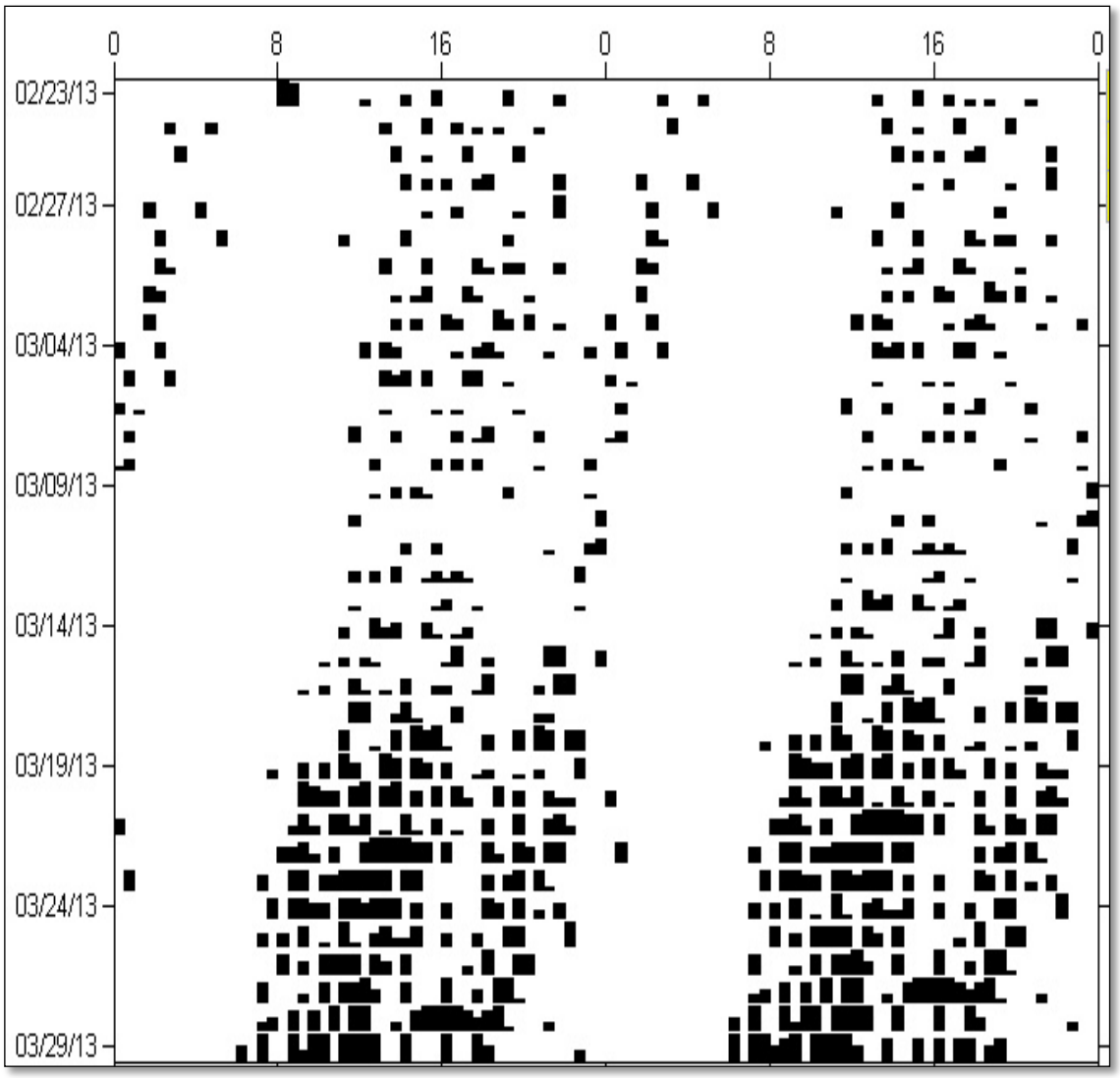

Figure 5. CIE-exposed mouse actogram. Thissample actogram shows the gradual acclimation to running-wheels in CIE treatment animals. The running-wheel activity is initially sparse and very sporatic. At the end of week three, their phase begins to shift on a similar slope to control animals. The first three weeks remain in a stationary phase. (Note: X-axis represents time in hours, with zero starting the next consecutive day).

DISCUSSION

The discovered results are consistent with previous results from the lab. As shown in figure 1, there was a significant effect of ethanol three-weeks after the initial withdrawal period from CIE treatment. The post-withdrawal symptoms have the possibility of being linked to lethargy or malaise. Furthermore, mice show very slight reductions in body weight and food intake when treated with chronic intermittent ethanol (Kliethermes et al, 2005). The ethanol-induced hypolocomotion suggests that ethanol-withdrawal state, as experienced by the CIE treated mice, may be analaogous to the depressive state that withdrawn human alcoholics may experience.

The acclimation to running-wheel activity is impaired in ethanol-exposed animals. Ethanol withdrawal is shown to induce hippocampal neurotoxicity (Stepanyan et al, 2008). The slow acclimation to running-wheel activity may be from the withdrawal-induced hippocampal function.

Rreduction in running-wheel activity may suggest that the CIE-exposed mice lack motivation in running. Running-wheel activity is intrinsically rewarding (Sherwin et al, 1998). It has antidepressant and anxiolytic properties (Duman et al, 2008; Salam et al, 2009). Moreover, it is genetically different from other ambulation methods (Rosenwasser et al, 1996). Therefore, the examination of running-wheel activity may provide a unique measure of reward-seeking behavior in animal studies of ethanol withdrawal.

There was no main effect of ketamine. Although ketamine slightly raised the locomotor activity in air-treated mice, it did not affect the CIE-exposed mce. Ketamine, in small doses, can induce a hyperlocomotive state (Irifune et al, 1991), seen in the not-

significant air animals. Ketamine did not alleviate the behavioral depression induced by alcohol withdrawal.

The failure of ketamine to alleviate these symptoms is surprising. Because alcohol withdrawal puts the brain in a hypersensitive state, it was expected that ketamine would relieve these symptoms by upregulating GABA. However, alcohol and ketamine both depress the central nervous sytem (Detsch and Kochs, 1997). The mechanism by which ketamine and alcohol interact is unknown. One may suggest that ketamine antagonizes the *N*-methyl-*D*-aspartate glutamate receptor, decreasing the levels of pre-synaptic glutamte, while alcohol withdrawal leaves NMDA receptors hypersensistive and with increased levels of the inhibitory neuron GABA.Ketamine is known to regulate AMPA/KA-glutamate receptors. This regulation is thought to be be the main mechanism of antidepressant treatment (Akinfiresoye and Tizabi, 2013; Maeng et al, 2008). Ethanol is a known AMPA-antagonist. Perhaps the combination of ethanol and ketamine antagonized AMPA and NMDA-receptors, resulting in no alleviation of the alcohol withdrawal induced depression.

In regard to chronobiology, ethanol alters the free-running period of mice. The ethanol- and ketamine-treated mice show and even short free-running period. Recent studies have shown that chronic ethanol intake alters free-running period in rodents (Brager et al, 2010; Rosenwasser et al, 2005a,b). The circadian disruptions may contribute to the behavioral depression seen. There is evidence that ketamine may play a role in modulating CLOCK genes *Bmal1, Per2,* and *Cry1* (Bellet et al, 2011). In our data, ketamine did not entrain the mice to a standard free-running period, suggesting that alternative mechanisms or interactions may be occuring. Ethanol-only treated animals do

not respond to circadian-entraining stimuli, such as light /dark cycles, as quickly as air animals do (see figures 4 and 5).

Overall, there was a main effect of alcohol on circadian rhythmicity and on behavioral depresion. This study repated our lab's previous findings that CIE-exposure and subsequent withdrawal induces a hypolocomotive state that can be translated intop protracted abstinence of human withdrawal stage. At this stage, human alcoholics are most likely to relapse in stressful situations (Heilig, 2010). Ketamine did not alleviate these symptoms, as hypothesized. Although ketamine is currently being used as an antidepressant in clinical human research, based upon this study, it should not be suggested as an antidepressant to relieve withdrawal symptoms.

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APPENDIX A

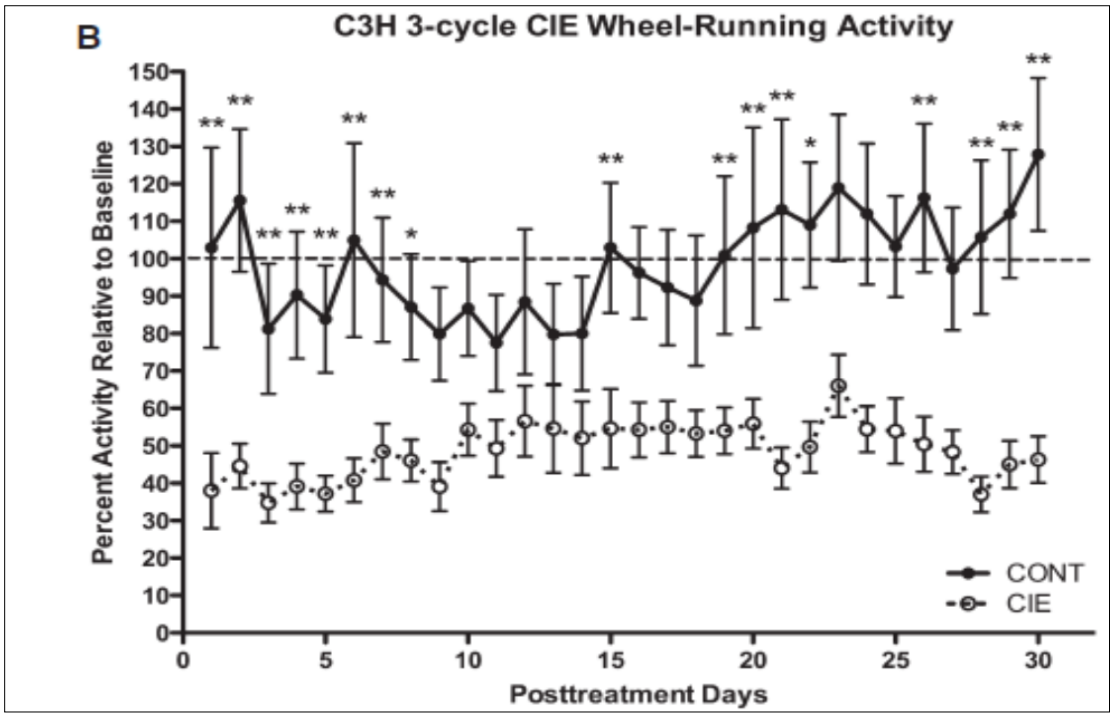

Chart A. C3H/HeJ CIE Wheel-Running Activity (Logan et al, 2012). C3H/HeJ mice exposed to CIE protocol show significantly decreased wheel-running activity up to four weeks post-treatment. The ethanol withdrawal induced hypolocomotion serves as a model analog for human behavioral depression (anhedonia).

APPENDIX B

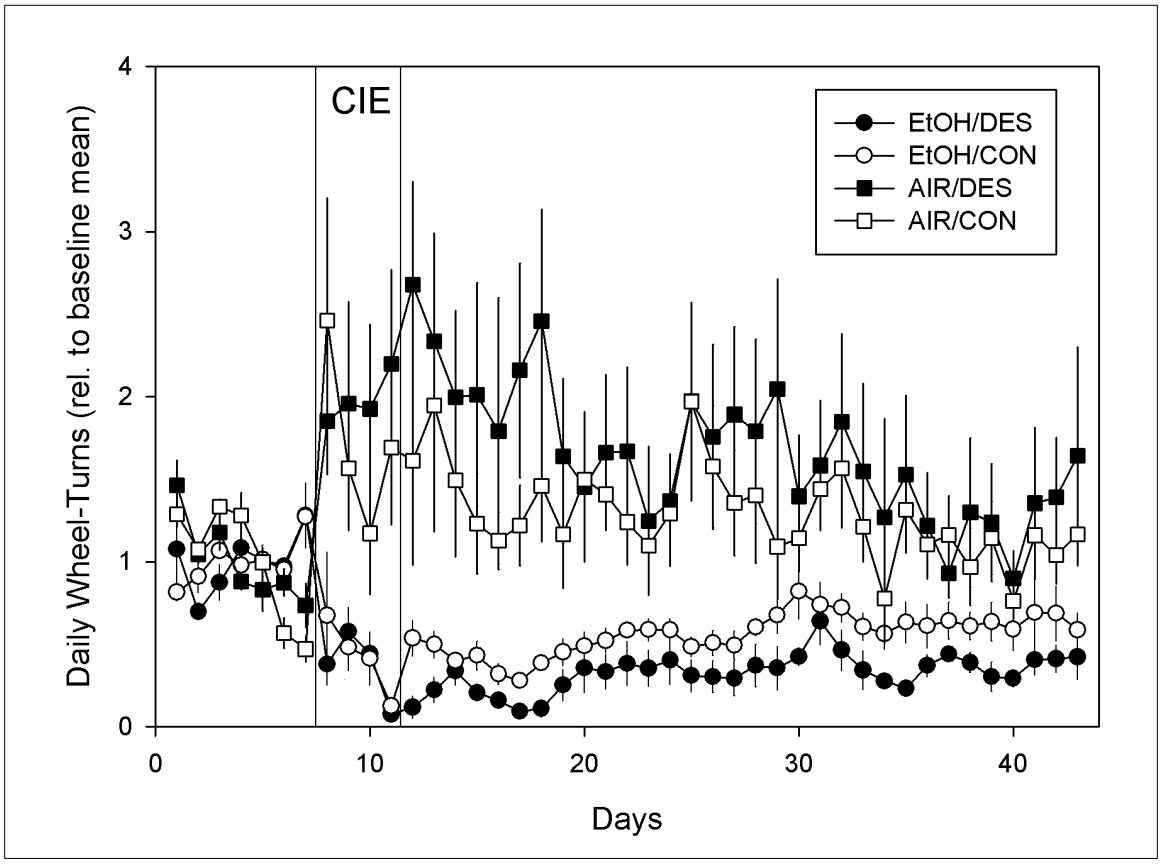

Chart B. Effects of desipramine administration following CIE protocol (Rosenwasser, unpublished). When administered with desipramine (i.p.) following traditional CIE protocol, C3H/HeJ mice showed no difference in running-wheel activity. The CIE treatment, and subsequent ethanol withdrawal, produces a hypolocomotion in running wheel activity, as expected.

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Christie M. Edwards was born in Springfield, Massachusetts on October 25, 1992. She was raised in Scarborough, Maine and graduated from Scarborough High School in 2010. Christie has a degree in biology, with a minor in neuroscience. She is actively involved in women's club volleyball, intramurals, and serves as a staff photographer for the Maine Campus. She has received a Center for Undergraduate Research fellowship and has presented research at the Maine Chapter of the Society for Neuroscience, Maine State House Undergraduate Research Day, and at the Maine Biological and Medical Sciences Symposium.

Upon graduation, Christie will be taking a gap year to work and travel, before attending medical school.